VERIFICATION OF TRANSLATION

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declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

2. That the attached document is a true and correct translation made by me to the best of my knowledge and belief of:-

(a) Japanese Patent Application No. Hei 9-260972
Entitled: " TRANSPORTER GENES "
Filed on September 8, 1997

September 5, 2000
(Date)

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【Name of Document】 APPLICATION FOR PATENT

【Identification Number】 C2-906

【Filing Date】 September 8, 1997

【Addressee】 Esq. Commissioner of the Patent Office

【International Patent Classification】 C12N 15/12

【Title of the Invention】 TRANSPORTER GENES

【Number of Claims】 12

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【Payment】

【Registration Number】 041092

【Amount】 21000

【List of Attached Documents】

【Name of Document】 Specification 1

【Name of Document】 Drawings 1

【Name of Document】 Abstract 1

【General Power of Attorney Number】 9710621

[Document Name] Specification

[Title of the Invention] Transporter Genes

[Claims]

[Claim 1] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

[Claim 2] A protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2.

[Claim 3] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

[Claim 4] A protein encoded by a DNA hybridizing to a DNA comprising nucleotide sequence set forth in SEQ ID NO: 4 and having an activity to transport an organic cation.

[Claim 5] A DNA encoding the protein according to claim 1 or 3.

[Claim 6] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2.

[Claim 7] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO:4.

[Claim 8] A vector comprising the DNA according to any one of claims 5 to 7.

[Claim 9] A transformant expressively carrying the DNA according to any one of claims 5 to 7.

[Claim 10] A method for producing the protein according to any one of claims 1 to 4, the method comprising culturing the transformant according to claim 9.

[Claim 11] An antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:1.

[Claim 12] An antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to transporters, proteins involved in transport of substances from the outside to the inside of cells or vice versa.

[0002]

[Prior Art]

Recently, the involvement of various transporters localized on the plasmamembrane in the uptake system for nutrients and endogenous substances into cells and their transport mechanisms have been clarified (Tsuji, A. and Tamai, I., Pharm. Res., 13, 963-977, 1996). These transporters recognize the structures of substances to be transported to selectively transport specific substances. Transporters that recognize the relatively wide range of structures may possibly recognize foreign substances such as drugs by mistake, and actively take in them into cells. It is believed that drugs permeate through the plasmamembrane fundamentally by simple diffusion depending on their physicochemical properties such as molecular size, hydrophobicity, and hydrogen-binding capacity. Particularly, in the case of ionic drugs, only molecules in the non-dissociated form can permeate through the plasmamembrane according to the pH partition hypothesis. However, it has become evident that a number of drugs penetrate through the cell membrane by a specific mechanism other than simple diffusion, that is, an active transport mediated by transporters, in organs that require efficient exchange of intracellular and extracellular substances, including small intestine, uriniferous tubule, placenta, epithelial cells of choroid plexus, hepatocytes, and blood-brain barrier (Tamai, I. and Tsuji, A., Pharmacia, 31, 493-497, 1995; Saito, H. and Inui, K., Igaku no Ayumi, 179, 393-397, 1996; Tamai, I., Yakubutsu Dotai (Pharmacokinetics), 11, 642-650, 1996). For example, it is known that although oral β-lactam antibiotics of the non-esterified type are amphoteric or negatively charged in physiological pHs and sparingly soluble in fat, they are readily absorbed through the intestine. The transport study

using the isolated membrane-vesicles system demonstrated that an H^{+}-driven peptide transporter localized on the brush-border is involved in the absorption process of these drugs (Okano, T. et al., J. Biol. Chem., 261, 14130-14134, 1986). Although the specificity of a peptide transport system in terms of the peptide size is so strict as to recognize di- or tri-peptides but not tetrapeptides or larger peptides, it has a rather broad substrate specificity to recognize peptides comprising non-natural amino acids. The peptide transporter mediates transport of β-lactam antibiotics mistakenly due to its broad substrate specificity. This property has been unexpectedly utilized in the clinical field (Tsuji, A., American Chemical Society (eds. Taylor, M. D., Amidon, G. L.), Washington, D. C., 101-134, 1995). Furthermore, a possibility that a transporter is also involved in permeation of substances with a high hydrophobicity such as fatty acids through the plasmamembrane has been reported (Schaffer, J. and Lodish, H., Cell, 79, 427-436, 1994).

[0003]

Since various transporters are supposed to be distributed in organs and cells based on the physiological roles of the organs and cells, their distribution and functions may be specific to organs. Therefore, transporters are expected to be used to impart an organ specificity to pharmacokinetics. In other words, an organ-specific drug delivery system (DDS) can be constructed utilizing transporters. If drug absorption solely relied on simple diffusion is improved by elevating its hydrophobicity, an effect of the drug obtained in the initial transport in the liver can be enhanced and the drug can non-specifically translocate into any organ. In addition, it would also be possible to increase the drug absorption independently of its fat-solubility by designing the drug utilizing the substrate specificity of transporters (Hayashi, K. et al., Drug Delivery System, 11, 205-213, 1996). For this purpose, it is necessary to identify various transporters at the molecular level and analyze their properties in detail. However, their molecular level identification are greatly behind studies on their membrane physiology because they

are difficult to handle biochemically and require complicated processes in their functional assays.

[0004]

Recently, cDNAs of several transporters have been cloned by the expression cloning method using *Xenopus* oocytes, a foreign gene expression system, and structural homology among them has been revealed (Fei, Y.-J. et al., Nature, 368, 563-566, 1994). For example, Koepsell et al. cloned an organic cation transporter, OCT1, which is assumed to be localized on a basement membrane, using the expression cloning method in 1994 (Grundemann, D. et al., Nature, 372, 549-552, 1994). Subsequently, OCT2 was identified by homology cloning based on the sequence of OCT1 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996). OCT1 and OCT2 show homology as high as 67% to each other, but they differ in their distribution at uriniferous tubules, and OCT2 is believed to be mostly localized at luminal side (Grundemann, D. et al., J. Biol. Chem., 272, 10408-10413, 1997). Both of them are intensely expressed in the kidney, but differ in the organ distribution; OCT1 is also expressed in the liver, colon, and small intestine, while OCT2 expression is specific to the kidney. It has been found that both NKT identified through screening kidney-specific genes (Lopez-Nieto, C.E. et al., J. Biol. Chem., 272, 6471-6478, 1997.) and NLT cloned as an antigen against an antibody that recognizes a membrane protein of hepatic sinusoid (Simonson, G. D. et al., J. Cell Sci., 107, 1065-1072, 1994.) show high homology with OCT1 and OCT2, and therefore, they might constitute one family (OCT family). Besides these, genes derived from *Drosophila* and *C. elegans* which show the same extent of homology are stored in the database (GenBank accession No. Y12400 and Z83228, respectively), and they are thought to be also included in this family. Thus, only a few reports on identification of transporters at the molecular level, including the reports, are available, and there would be many unknown transporters that may be clinically useful.

[0005]

[Problems to be Solved by the Invention]

An object of this invention is to provide a novel transporter.

[0006]

[Means to Solve the Problems]

The present inventors have screened a fetal gene library constructed using the subtractive method by random sequencing based on a working hypothesis that fetal genes include those which are involved in various disorders including cancer and are specifically or intensely expressed in fetal tissues. The inventors discovered an unknown gene showing a significant homology with those for organic cation transporter family proteins recent-cloned. As a result of cloning the gene and analyzing its structure, the inventors found that the gene encodes a novel protein which in overall shows high homology with organic cation transporter family proteins. Furthermore, the inventors studied the transporter activity of a protein encoded by the isolated gene and found that the protein, in fact, functioned as a transporter for various organic cations.

[0007]

This invention relates to a novel transporter, and more specifically to:

(1) a protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1, or a protein comprising said amino acid sequence in which one or more amino acid residues are substituted, deleted, or added, and having an activity to transport an organic cation;

(2) a protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2 and having an activity to transport an organic cation;

(3) a protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3, or a protein comprising said amino acid sequence in which one or more amino acid residues are substituted, deleted, or added, and having an activity to transport an organic cation;

(4) a protein encoded by a DNA hybridizing to a DNA comprising nucleotide sequence set forth in SEQ ID NO: 4 and having an activity to transport an organic cation;

(5) a DNA encoding the protein according to (1) or (3);

(6) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2 and encoding the protein having an activity to transport an organic cation;

(7) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO:4 and encoding the protein having an activity to transport an organic cation;

(8) a vector comprising the DNA according to any one of (5) to (7);

(9) a transformant expressively carrying the DNA according to any one of (5) to (7);

(10) a method for producing the protein according to any one of (1) to (4), the method comprising culturing the transformant according to (9);

(11) an antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:1; and

(12) an antibody that binds to the protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[0008]

[Mode for Carrying Out the Invention]

This invention relates to a novel transporter protein. Nucleotide sequences of cDNAs of novel human transporters isolated by the present inventors are shown in SEQ ID NO: 2 and SEQ ID NO: 4, respectively. Amino acid sequences of proteins encoded by these cDNAs are shown in SEQ ID NO: 1 and SEQ ID NO: 3, respectively. Amino acid sequences of these two proteins included in the transporter proteins of this invention showed such a high overall homology as about 72%, and both of them retained the following consensus sequence which is conserved in various types of transporters including the glucose transporter: [Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala] (Maiden, M. C. et al., Nature,

325, 641-643, 1987). In fact, proteins set forth in SEQ ID NO:1 have an activity to transport various organic cations.

[0009]

Transporter proteins of this invention also include those having the additional activity to transport substances other than organic cations as far as they retain the organic cation transport activity. Organic cations include, for example, TEA, carnitine, quinidine, and pyrilamine. Transporter proteins of this invention include those having the activity to transport organic cations not only from the outside to the inside of cells but also from the inside to the outside of cells.

[0010]

Transporter proteins of this invention can be prepared as recombinant proteins using recombination techniques or natural proteins. Recombinant proteins can be prepared, for example, as described below, by culturing cells transformed with DNA encoding proteins of this invention. Natural proteins can be isolated from the kidney and cancer cell strains such as Hela S3, which highly express the proteins of this invention, by the method well known to those skilled in the art, for example, affinity chromatography using an antibody of this invention described below. The antibody may be either polyclonal or monoclonal. A polyclonal antibody can be prepared by purifying serum obtained from, for example, a small animal such as a rabbit immunized with proteins of this invention by known methods, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. A monoclonal antibody can be prepared by immunizing a small animal such as a mouse with the protein of this invention, excising the spleen from the mouse, grinding the tissue into cells, fusing them with mouse myeloma cells using a fusing agent such as polyethylene glycol, and selecting a clone that produces an antibody to proteins of this invention out of fused cells (hybridomas) thus produced. Then, hybridomas thus selected are transplanted into the abdominal cavity of a mouse, and the ascites is collected from the mouse. A monoclonal antibody

thus obtained can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. When the antibody thus obtained is administered to human subjects, a humanized antibody or a human antibody is advantageously used to reduce the immunogenicity. An antibody can be humanized by, for example, the CDR grafting method comprising cloning an antibody gene from monoclonal antibody-producing cells and grafting the epitope portion thereof into an existing human antibody. A human antibody can be prepared by the usual method for preparing a monoclonal antibody except for immunizing a mouse whose immune system is replaced with the human's.

[0011]

It is also possible for those skilled in the art to prepare proteins having functions substantially equivalent to the transporter proteins by appropriately modifying amino acid residues of the proteins set forth in SEQ ID NOs: 1 or 3 by, for example, substitution, using well known methods. Mutation of amino acids of the proteins can occur also spontaneously. Such mutant proteins which are obtained by altering the amino acid sequence of the transporter proteins set forth in SEQ ID NOs: 1 or 3 of this invention by substitution, deletion, or addition of amino acid residues, and have an activity to transport organic cations are also included in the proteins of this invention. Methods well known to those skilled in the art for altering amino acids include, for example, the site-specific mutagenesis system by PCR (GIBCO-BRL, Gaithersburg, Maryland), site-specific mutagenesis by oligonucleotide (Kramer, W. and Fritz, H. J. (1987) Methods in Enzymol., 154: 350-367), Kunkel's method (Methods Enzymol., 85, 2763-2766 (1988)), etc. The number of amino acids that can be substituted is usually 10 amino acid residues or less, preferably 6 or less, and more preferably 3 or less. The site of substitution, deletion, or addition of amino acid residues is not particularly limited as far as the activity of proteins of this invention is retained. It is possible to detect the transporter activity of

proteins, for example, by the method described below in Example 6.

[0012]

It is routine for those skilled in the art to obtain proteins which their function is substantially equivalent to those of the proteins of this invention by isolating and using DNAs highly homologous to the DNA sequences set forth in SEQ ID NOs: 2 or 4 or portions thereof using hybridization techniques (Sambrook, J. et al., Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. press, 1989), etc. Therefore, those skilled in the art can prepare proteins which are encoded by a DNA hybridizing to a DNA set forth in SEQ ID NOs:2 or 4 and have an activity to transport organic cations. These proteins are also included in proteins of this invention. DNAs that hybridize to the DNAs encoding the proteins of this invention can be isolated from other organisms, for example, mice, rats, rabbits, cattle, etc. Especially, tissues such as the kidney are suitable as sources of such DNAs. These DNAs which were isolated using hybridization techniques and encode the proteins having substantially equivalent function to the proteins set forth in SEQ ID NOs: 1 or 3 usually have a high homology with the DNAs set forth in SEQ ID NOs: 2 or 4. "High homology" means at least 70% or more, preferably at least 80% or more, and more preferably at least 90% or more of sequence identity with DNAs set forth in SEQ ID NOs: 2 or 4.

[0013]

One example of hybridization conditions for isolating such DNAs is as follows. That is, after the pre-hybridization at 55℃ for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 37℃ to 55℃ for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS once at 37℃ for 20 min. More preferable conditions are as follows. After the pre-hybridization at 60℃ for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed

by heating the reaction mixture at 60℃ for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS twice at 50℃ for 20 min. Still more preferable conditions are as follows. After pre-hybridization at 68℃ for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 68℃ for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 0.1 x SSC and 0.1% SDS twice at 50℃ for 20 min.

[0014]

The present invention also relates to DNAs encoding the above-described transporter proteins of this invention. DNAs of this invention may be cDNA, genomic DNAs, and synthetic DNAs. The DNAs of the present invention can be used for producing proteins of this invention as recombinant proteins, as well as for isolating proteins which their function is substantially equivalent to those of proteins set forth in SEQ ID NOs:1 or 3 . That is, it is possible to prepare proteins of this invention as recombinant proteins by inserting DNAs encoding proteins of this invention (e.g. DNAs set forth in SEQ ID NOs: 2 or 4) into an appropriate expression vector, culturing transformants obtained by transfecting suitable cells with the vector, and purifying the proteins thus expressed. Cells to be used for producing recombinant proteins include, for example, mammalian cells such as COS cells, CHO cells, NIH3T3 cells, etc., insect cells such as Sf9 cells, yeast cells, *E. coli*, and so on. Vectors used for the intracellular expression of recombinant proteins vary depending on host cells, including, for example, pcDNA3 (Invitrogen), pEF-BOS (Nucleic Acids Res., 1990, 18(7), p5322), etc. for mammalian cells, "BAC-to-BAC baculovirus expression system" (GIBCO BRL), etc. for insect cells, "Pichia Expression Kit" (Invitrogen), etc. for yeast cells, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), etc. for *E. coli*. Host cells can be transformed with vectors, for example, by the calcium phosphate method, the DEAE-dextran method, the method using

cationic liposome DOTAP (Boehringer Mannheim), the electroporation method, the calcium chloride method, etc. Recombinant proteins can be purified from recombinants thus obtained using standard methods, for example, as described in "The Qiaexpressionist Handbook, Qiagen, Hilden, Germany."

[0015]

The DNAs of this invention can be used in gene therapy for disorders caused by abnormalities in the activity and expression of the proteins of this invention. In this case, the DNAs of this invention are inserted into an adenovirus vector (e.g. pAdexLcw), a retrovirus vector (e.g. pZIPneo), etc., and administered into the body by either *ex vivo* method or *in vivo* method. Gene therapy can also be performed by administering a synthetic antisense DNA to the body either directly or after inserting into the above-described vector.

[0016]

The transporter proteins of this invention can be used to control internal absorption and dynamics of drugs. Based on the results of detailed analysis of the substrate specificity of transporter proteins of this invention, drugs can be designed so as to be transported by these transporters and absorbability of the drugs mediated by these transporter proteins can be improved. Conventional modifications to enhance hydrophobicity are no longer necessary for drugs so designed, which enables speedily and efficiently developing water-soluble drugs that are easy to handle. The drugs thus developed are thought to be absorbed principally depending on the internal distribution pattern of transporter proteins of this invention, and an organ-specific delivery of the drugs thus becomes possible. Especially, if the transporter proteins of this invention are distributed in the target organ of a drug, an ideal drug delivery system (DDS) can be developed. If a drug is to be absorbed mediated by not the transporter proteins of this invention but other transporters, the drug can be designed so as to be specific to other transporter proteins by designing it considering the substrate specificity of the transporter proteins of this invention. Since the transporter proteins of

this invention are present in the kidney, it is possible to reduce the nephrotoxicity produced by a drug by designing the drug so that it can be readily excreted by the transporter proteins of this invention.

[0017]

Another possible application of this invention is to develop a drug targeting the transporter proteins of this invention. The transporters play important roles in the absorption mechanism of nutrients and drugs, or the excretion mechanism of drugs and internal metabolites. Thus, damage or abnormal elevation of the transporter's functions may cause some disorders. It is considered to be efficacious against such disorders to administer a drug containing a compound that inhibits or enhances functions of the transporter proteins of this invention, or regulates the expression level of the transporter gene of this invention and the amount of the transporter proteins. Also, the above-mentioned gene therapy will be efficacious.

[0018]

The transporter proteins of this invention are expressed in a variety of cancer cell strains, which suggests that the proteins may transport drugs into tumor cells. If this is the case, it is possible to develop carcinostatics that will be readily absorbed mediated by the transporter proteins of this invention. On the contrary, mechanisms to transport and excrete substances by the transporter proteins of this invention may function to excrete carcinostatics in tumor cells so that the cells acquire resistance to drugs. If the transporter proteins of this invention are involved in a mechanism of tumor cells to acquire drug resistance, a carcinostatic effect can be enhanced by using inhibitors of the transporter proteins of this invention.

[0019]

The present invention is described below in more detail with reference to examples, but is not construed being limited thereto.

[0020]

[Examples]

Example 1 Construction of a subtraction library

A subtraction library was constructed using the PCR-Select™ cDNA Subtraction Kit (CLONTECH) principally according to the method of Luda Diatchenko (Diatchenko, L. et al., Proc. Natl. Acad. Sci. USA, 93, 6025-6030, 1996).

[0021]

First, double-stranded cDNAs were synthesized from poly(A)$^+$ RNAs derived from human fetal liver and adult liver by the standard method using MMLV reverse transcriptase. These cDNAs were blunt-ended with T4 DNA polymerase and cleaved with RsaI. A part of the cDNAs derived from fetal liver (tester) was divided in two portions, and they were separately ligated to two different adapters, adapter 1 and adapter 2, respectively (Table 1). A 120-fold excess of cDNA derived from adult liver (driver) was added to each of the above-described tester samples. The mixture was heat-denatured and subjected to the primary hybridization at 68℃ for 8 h. After these two reaction mixtures from the primary hybridization were mixed together without heat-denaturation, an excessive amount of the heat-denatured driver was further added thereto, and the mixture was subjected to the secondary hybridization at 68℃ for about 16 h. The resulting reaction solution was diluted with a dilution buffer and incubated at 75℃ for 7 min. After the shorter strands of adapters were removed, the reaction solution was used as a template for PCR. PCR using primers 1 (5'-CTAATACGACTCACTATAGGGC-3', SEQ ID NO: 5) and 2 (5'-TGTAGCGTGAAGACGACAGAA-3', SEQ ID NO: 6) corresponding to the adapters selectively amplified only cDNAs having different adapters at their both ends (subtracted cDNAs)(suppression PCR). PCR was carried out using a portion of the resulting cDNA as a template, and nested PCR primers 1 (5'-TCGAGCGGCCGCCCGGGCAGGT-3', SEQ ID NO: 7) and 2 (5'-AGGGCGTGGTGCGGAGGGCGGT-3', SEQ ID NO: 8), which are further inwardly located from the PCR primers 1 and 2, to obtain products with further elevated selectivity. PCR products thus obtained were purified using the QIAquick PCR Purification kit (QIAGEN), and cloned into the pT7Blue-T vector (Novagen) by the TA cloning method to construct a subtraction library.

[0022]

[Table 1]

Adapter 1	5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT-3' 3'-GGCCCGTCCA-5'
Adapter 2	5'-TGTAGCGTGAAGACGACAGAAAGGGCGTGGTGCGGAGGGCGGT-3' 3'-GCCTCCCGCCA-5'

Example 2 cDNA cloning

To analyze fetal genes, the subtraction library derived from the fetal liver was screened by random sequencing. Homology search (Blastx) of Expressed Sequence Tags (ESTs) thus obtained found a clone, fls631(292 bp) encoding amino acid sequence having significant homology with the known organic cation transporters, OCT1 (Grundemann, D. et al., Nature, 372, 549-552, 1994), OCT2 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996), and their family proteins(OCT family protein). Since the sequence of this clone was novel and assumed to be a fragment derived from a new transporter gene, cDNA comprising the whole open reading frame (ORF) of this gene was cloned.

[0023]

The human fetal liver 5'-stretch cDNA library (CLONTECH) was screened using the original fls631 clone obtained from the subtraction library derived from fetal liver as a probe. An insert of the original fls631 clone was amplified by PCR using M13 P4-22 and M13 P5-22, and labeled with [α-^{32}P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was carried out at 68℃ in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50℃ in 0.1 x SSC and 0.1% SDS. Screening about 5 x 10^5 phage clones finally isolated seven positive clones. cDNA inserts of these clones were amplified by PCR using vector primers designed based on a sequence of the λgt10 vector (GT10 S1 5'-CTTTTGAGCAAGTTCAGCCT-3', SEQ ID NO: 9, and GT10 A1 5'-AGAGGTGGCTTATGAGTATTTCTT-3', SEQ ID NO: 10), or primers designed based on the decoded cDNA sequences. The PCR products thus obtained were directly sequenced to determine the nucleotide sequences. Some regions that were difficult to be amplified were

subjected to PCR using 7-deaza dGTP as a substrate base (McConlogue, L. et al., Nucleic Acids Res., 16, 9869, 1988).

[0024]

After screening about 5 x 10^5 phage clones, finally seven positive clones were isolated. Sequencing of cDNA inserts of these clones revealed that the fls631 gene contains an ORF encoding a protein consisting of 551 amino acid residues (putative molecular weight of about 62,000). Data base search using this whole amino acid sequence confirmed that it has a significant homology not only with the region corresponding to the original fls631 clone but also in overall with known OCT family proteins. Figure 1 shows a comparison between amino acid sequences of fls631 and OCT family proteins (rat OCT1, rat OCT2, mouse NKT(Lopez-Nieto, C. E. et al., J. Biol. Chem., 272, 6471-6478, 1997.), and rat NLT(Simonson, G.D., et al., J. Cell Sci., 107, 1065-1072, 1994.)). The fls631 showed high homology at the amino acid level to these family proteins, being 32%, 33%, 28%, and 28%, respectively. Hydrophobicity profile of this sequence obtained by Kyte & Doolittle's calculating formula (Kyte, J. and Doolittle, R. F., J. Mol. Biol., 157, 105-132, 1982) very closely resembled those of OCT family, indicating that the sequence has eleven to twelve putative transmembrane hydrophobic regions (Fig. 2). This sequence contained one consensus sequence of sugar transporter, ([Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala]), (160 to 175). This consensus sequence is present in the glucose transporters GLUT1 to GLUT7 in mammalian cells, and also present in various types of transporters other than glucose transporters (Maiden, M. C. et al., Nature, 325, 641-643, 1987). Furthermore, putative N-linked glycosylation sequences (N-X-[ST]) were found in the amino acid sequence of fls631 at four sites (57 to 59, 64 to 66, 91 to 93, and 304 to 306). Though possibility of modification by N-linked sugar chain has been suggested for the OCT family, it has not been biochemically proved, and its biological significance has not been clarified yet. And there were five

putative protein kinase C phosphorylation sites ([ST]-X-[RK]) (164 to 166, 225 to 227, 280 to 282, 286 to 288, and 530 to 532). Among them, particularly the 286-288 site among them was conserved at a common position in all OCN family proteins. In addition, the consensus sequence ([Ala, Gly]-Xaa(4)-Gly-Lys-[Ser, Thr]) of the ATP/GTP binding site is also found. This consensus sequence of the ATP/GTP binding site is also present in the ATP binding protein or GTP binding protein, such as kinases and ras family proteins, and that ATP or GTP binds to this site (Walker, J. E. et al., EMBO J., 1, 945-951, 1982). This sequence is present in the so-called ATP Binding Cassette (ABC) type transporter, and involved in the substance transport using the energy generated by hydrolysis of ATP (Higgins, C. F. et al., J. Bioenerg. Biomembr., 22, 571-592, 1990; Urbatsch, I. L. et al., J. Biol. Chem., 270, 26956-26961, 1995). Presence of this consensus sequence indicates that fls631 protein may be an ATP or GTP-dependent transporter.

[0025]

Nucleotide sequencing was performed by the cycle-sequencing method with a plasmid DNA prepared by the alkaline-SDS method or a PCR product obtained by colony PCR, etc. as a template using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit With AmplyTaq DNA Polymerase, FS, followed by decoding with the ABI 377 DNA Sequencer (Perkin Elmer). Colony PCR was carried out by directly suspending a colony of a recombinant in a PCR reaction solution containing vector primers M13 P4-22 (5'-CCAGGGTTTTCCCAGTCACGAC-3', SEQ ID NO: 11) and M13 P5-22 (5'-TCACACAGGAAACAGCTATGAC-3', SEQ ID NO: 12). After the completion of PCR, a DNA insert thus amplified was separated from unreacted primers and nucleotides by gel filtration, etc. to serve as a template for sequencing.

[0026]

Example 3 Northern analysis

Distribution of fls631 in tissues was investigated by Northern analysis (Fig. 3). A 3'-end fragment of fls631 (the latter half from around the base 1,100) was labelled with [α-^{32}P]dCTP by the random primer method using the Ready-to Go DNA labeling beads

(Pharmacia) to serve as a probe. Hybridization was performed using the Multiple Tissue Northern (MTN) Blot - Human, Human III, Human IV, Human Fetal II, and Human Cell lines (CLONTECH) at 68℃ in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50℃ in 0.1 x SSC and 0.1% SDS. As a result, RNA of about 2.5 kb was strongly expressed in the fetal liver and adult-derived tissues such as the kidney, bone marrow, and trachea. Besides those tissues, the RNA band was also weakly detected in the fetal kidney and lung, and adult tissues including skeletal muscle, lung, placenta, prostate, spleen, and spinal cord. The RNA expression was also detected in tumor cell lines such as HeLa S3, K562, SW480, and A549, and especially, its very intense expression was observed in HeLa S3.

[0027]

Example 4 Cloning of 631RT cDNA

Data base search using the entire nucleotide sequence of "fls631" detected very similar sequences thereto in several parts of the nucleotide sequence of P1 phage clones (P1 H24 clones, GenBank accession No. L43407, L43408, L46907, L81773, and L43409) derived from q regions of human chromosome 5 reported at Human Genome Center. The parts having similarity with the nucleotide sequence of fls631 but being obviously different from it are included. The similar sequence are separated by the sequences regarding as intron. The sequence obtained by connecting these similar parts with each other with reference to the sequence of OCTN1 has a high homology over a wide range with fls631(Fig 4), indicating the presence of fls631 homologues like OCT1 and OCT2 relationship. Therefore, in order to perform cDNA cloning of this homologous gene, 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631R A1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) were prepared based on sequences of these P1 phage clones. PCR was performed using a set of these primers and cDNA synthesized from poly(A)$^+$ RNA derived from the human adult kidney (CLONTECH) as a template, under the following conditions: 1 cycle of 94℃ for 3 min; 35 cycles of94℃ for 30 s, 58℃ for 1 min, and 72℃ for 2 min,; and 1 cycle

of 72℃ for 10 min, resulting in amplification of about 900 bp fragment. This fragment was subcloned into the pT7Blue-T vector (Novagen) by the TA cloning method to determine its nucleotide sequence, which clearly showed a very high overall homology with fls631. Therefore, this gene was designated as 631RT (fls 631 Related Transporter), and longer cDNAs were cloned.

[0028]

Renewably, the 631R S6 primer (5'-AGCATCCTGTCTCCCTACTTCGTT-3', SEQ ID NO: 15) was prepared. Cloning was performed by the Rapid Amplification of cDNA Ends (RACE) method (Chenchik, A., Moqadam, F., and Siebert, P. (1995), CLONTECHniques X, 5-8). PCR was performed using this primer and the Marathon-Ready™ cDNA derived from the human adult kidney (CLONTECH) as a template under the following conditions: 94℃ for 2 min; 35 cycles of 94℃ for 30 s, 60℃ for 1 min, and 72℃ for 3 min,; and 72℃ for 10 min, resulting in amplification of about 1.7 kbp cDNA fragment of the 3'-end. This fragment was subcloned into the pT7Blue-T vector by the TA cloning method. The partly nucleotide sequence determination of the clone revealed that it was a fragment of the 3'-end of 631RT. The sequence obtained by these methods was shown in SEQ ID NO: 3.

[0029]

It became evident that the sequence contains an open reading frame (ORF) encoding a protein consisting of 460 amino acid residues. Fig. 5 compares amino acid sequences of the ORF and fls631. Both showed overall amino acid homology as high as about 72%. In addition, one consensus sequence of sugar transporter was present in the amino acid sequence of 631RT like fls631. These facts indicated that 631RT can be a novel transporter that is structurally related to fls631. Furthermore, a consensus sequence of the ATP/GTP binding site was also present in the amino acid sequence of 631RT like fls631. The 631RT cDNA comprising a longer region could be cloned using the cDNA already obtained as a probe in the same manner as for fls631 cDNA cloning.

[0030]

Example 5 Northern analysis

Northern analysis was performed using about 900 bp 631RT cDNA as a probe which was obtained by PCR with a set of 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631R A1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) in the same manner as for fls631. The results are shown in Fig. 6. Although the expression pattern of 631RT partly overlapped with that of fls631, 631RT differs from fls631 in that the former was very intensely expressed in the kidney among fetal tissues, while 631RT was strongly expressed also in cancer cell strains such as K-562, HeLa S3, SW480, etc. as well as the kidney, indicating that fls631 and 631RT may be involved in transport of substances such as carcinostatics in these cancer cells.

[0031]

Example 6 Forced expression of fls631 in human fetal kidney cells (HEK293) and its activity determination

Phage DNAs were extracted from positive phage clones obtained by screening the clones by the plaque hybridization method using the QIAGEN Lambda Kit (QIAGEN). After the DNA insert was subcloned into the pUC18 vector, cDNA containing the entire ORF which was cleaved out with SmaI and EcoRI was integrated between the EcoRI site and the blunted HindIII site of an expression vector for mammalian cells, pcDNA3 (Invitrogen), to obtain an expression plasmid DNA, pcDNA3/631-2. Plasmid DNA was prepared by alkaline-SDS method using the QIAGEN PLASMID MAXI Kit (QIAGEN).

[0032]

The human fetal kidney-derived cell strain, HEK 293 cells were transfected with the plasmid pcDNA3/631-2 and pcDNA3 vector containing no insert as a control by the calcium phosphate method. First, the plasmid DNA (10 μg), a Hepes buffer solution (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM Dextrose, and 21 mM Hepes pH 7.1) (1 ml), and 2M $CaCl_2$ (62.5 μl) were combined and allowed to stand at room temperature for 30 min or more to form calcium phosphate coprecipitates. After cells were plated on 10-cm plates at 1.5 x 10^6 cells per plate and cultured for 24 h, the calcium phosphate coprecipitates were added thereto, and the cells were further cultured for 24 h. Then, plates were washed with phosphate buffered

saline (PBS), and the cells were further cultured for 24 h after the addition of fresh culture medium.

[0033]

Transport experiment was performed using cells transfected with the plasmid DNA or untreated cells according to the following procedures. Cells were detached from plates using a rubber policeman, suspended in a transport buffer (containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM (+)-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 25 mM Hepes pH 7.4), and pre-incubated for 20 min. An appropriate amount of each labeled substrate ([^{14}C]TEA (tetraethylammonium) (NEN), [^{3}H]carnitine (L-carnitine hydrochloride) (Amersham), [^{3}H]PCG (benzylpenicillin) (Amersham), [^{3}H]quinidine (ARC), or [^{3}H]pyrilamine (mepyramine) (Amersham)) was then added to the cell suspension, and the resulting mixture was incubated at 37℃ for a predetermined period of time. Incubated cells were overlaid on a silicon layer prepared by layering a mixture of silicon oil and liquid paraffin (specific gravity = 1.022) on a 3 M KCl layer, and separated by centrifugation. Radioactivity of cells was measured to determine the into-the-cell transport activity. In this case, 1 x 10^6 cells were used as one point of cells. HEK 293 cells were cultured in Dulbecco's MEM containing 10% fetal calf serum (FCS) in an atmosphere of 5% carbon dioxide at 37℃.

[0034]

First, the transporter capacity was measured in the cells transfected with pcDNA3/fls631-2 and untreated cells using TEA as a substrate (Fig. 7). A reaction time-dependent TEA uptake into the fls631-transfectd cells was clearly observed. This uptake was not observed in untreated cells. Next, effects of the addition of unlabeled TEA on the labeled substrate uptake in this system (cold inhibition) were examined (Fig. 8). A decrease in the apparent uptake of the labeled substrate was clearly seen depending on the concentration of cold TEA added. In this experiment, almost no uptake of the substrate into cells was observed in cells transfected with the pcDNA3 vector containing no insert (Mock) used as a control like in untreated cells used, clearly indicating

that this uptake phenomenon is due to the transfection of the cells with fls631. Next, to obtain the Km (Michaelis constant) value of fls631 to TEA, the uptake of ^{14}C-TEA with various concentrations was measured (Fig. 9). From Lineweaver-Burk reciprocal plot of the net uptake obtained by subtracting the amount of the uptake in Mock cells from that in the human fls631-transfected cells, the Km value of 0.44 ± 0.04 mM was obtained with the maximal velocity, Vmax of 6.68 ± 0.34 (nmol/3 min/mg). Next, the transport capacity of OCTN1 for other substrate than TEA was examined (Fig. 10). When the transport capacity was measured using labeled organic cations such as labeled carnitine, quinidine, and pyrilamine, a significant increase in the uptake of these compounds was clearly observed in fls631-transfected cells as compared with Mock cells, clearly indicating that these organic cations can serve as substrates for fls631. However, no significant increase in the uptake of an organic anion, PCG (benzylpenicillin), was observed.

[0035]

[Effect of the Invention]

This invention provides a novel organic cation transporter. Transporter of this invention are useful for developing newly designed drugs that can be transported mediated by these proteins, and pharmaceuticals for disorders caused by functional abnormalities of the proteins.

[Sequence Listing]
SEQ ID NO: 1
LENGTH: 551
TYPE: amino acid
TOPOLOGY: linear
MOLECULE TYPE: protein
SEQUENCE

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 10                 15                  20                  25
Ser Ala Ser Ile Ile Pro Asn Gly Phe Asn Gly Met Ser Val Val Phe
                30                  35                  40
Leu Ala Gly Thr Pro Glu His Arg Cys Arg Val Pro Asp Ala Ala Asn
            45                  50                  55
Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp
        60                  65                  70
Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile
    75                  80                  85
Ala Asn Phe Ser Ala Leu Gly Leu Glu Pro Gly Arg Asp Val Asp Leu
90                  95                  100                 105
Gly Gln Leu Glu Gln Glu Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln
                110                 115                 120
Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu
            125                 130                 135
Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val
        140                 145                 150
Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg
    155                 160                 165
Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe
170                 175                 180                 185
Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val
                190                 195                 200
Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu
            205                 210                 215
Gly Thr Glu Ile Leu Gly Lys Ser Val Arg Ile Ile Phe Ser Thr Leu
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220 225 230
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235 240 245
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250 255 260 265
Pro Gly Val Leu Cys Val Pro Leu Trp Trp Phe Ile Pro Glu Ser Pro
270 275 280
Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile
285 290 295
Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe
300 305 310
Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile
315 320 325
Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser
330 335 340 345
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365 370 375
Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg
380 385 390
Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly
395 400 405
Gly Val Leu Leu Phe Ile Gln Leu Val Pro Val Asp Tyr Tyr Phe Leu
410 415 420 425
Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe
430 435 440
Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
445 450 455
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460 465 470
Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
475 480 485
Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
490 495 500 505
Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln

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525 530 535
Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
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SEQ ID NO: 2
LENGTH: 2135
TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULE TYPE: cDNA to mRNA
FEATURE
NAME/KEY: CDS
LOCATION: 147..1799
DEFINITION METHOD: E
SEQUENCE

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CAAGTTTCGG AGCGGCAGTG GGAAGC ATG CGG GAC TAC GAC GAG GTG ATC GCC    173
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AGC GCC AGC ATC ATC CCC AAT GGC TTC AAT GGT ATG TCA GTC GTG TTC     269
Ser Ala Ser Ile Ile Pro Asn Gly Phe Asn Gly Met Ser Val Val Phe
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             45                  50                  55
CTG AGC AGC GCC TGG CGC AAC AAC AGT GTC CCG CTG CGG CTG CGG GAC     365
Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp
         60                  65                  70
GGC CGC GAG GTG CCC CAC AGC TGC AGC CGC TAC CGG CTC GCC ACC ATC     413
Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile
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Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu

125 130 135

GAC AAC TGG AAG GTG CCC CTC ACC ACC TCC CTG TTC TTC GTA GGC GTG 605

Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val

140 145 150

CTC CTC GGC TCC TTC GTG TCC GGG CAG CTG TCA GAC AGG TTT GGC AGG 653

Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg

155 160 165

AAG AAC GTT CTC TTC GCA ACC ATG GCT GTA CAG ACT GGC TTC AGC TTC 701

Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe

170 175 180 185

CTG CAG ATT TTC TCC ATC AGC TGG GAG ATG TTC ACT GTG TTA TTT GTC 749

Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val

190 195 200

ATC GTG GGC ATG GGC CAG ATC TCC AAC TAT GTG GTA GCC TTC ATA CTA 797

Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu

205 210 215

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220 225 230

GGA GTG TGC ACA TTT TTT GCA GTT GGC TAT ATG CTG CTG CCA CTG TTT 893

Gly Val Cys Thr Phe Phe Ala Val Gly Tyr Met Leu Leu Pro Leu Phe

235 240 245

GCT TAC TTC ATC AGA GAC TGG CGG ATG CTG CTG CTG GCG CTG ACG GTG 941

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GGT GTG CTT CTC TTC ATT CAA CTG GTA CCT GTG GAT TAT TAC TTC TTA      1421
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Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
             445                      450                      455
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CATTTCCAAT CATACAAATA CTATCCAAAT AAAAAT 2135

SEQ ID NO: 3
LENGTH: 460
TYPE: amino acid
TOPOLOGY: linear
MOLECULE TYPE: protein
SEQUENCE
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20 25 30
Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro Leu
35 40 45
Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile Ser

50 55 60
Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Val Thr
65 70 75
Met Gly Thr Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys Asn
80 85 90 95
Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln Ile
100 105 110
Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly Lys
115 120 125
Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr Ala
130 135 140
Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp Trp
145 150 155
Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val Ala
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Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala Asn
195 200 205
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210 215 220
Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu Arg
225 230 235
Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp Met
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Thr Leu Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn Leu
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Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg Arg
290 295 300
Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu Phe
305 310 315
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320 325 330 335
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340 345 350
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385 390 395
Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu Ser
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Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys Asp
435 440 445
Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe
450 455 460

SEQ ID NO: 4
LENGTH: 1555
TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULE TYPE: cDNA to mRNA
SEQUENCE
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TGT CTG GAT GGC TGG GAG TTC AGT CAG GAC GTC TAC CTG TCC ACC ATT 95
Cys Leu Asp Gly Trp Glu Phe Ser Gln Asp Val Tyr Leu Ser Thr Ile
20 25 30
GTG ACC GAG TGG AAC CTG GTG TGT GAG GAC GAC TGG AAG GCC CCA CTC 143
Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro Leu
35 40 45
ACA ATC TCC TTG TTC TTC GTG GGT GTG CTG TTG GGC TCC TTC ATT TCA 191
Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile Ser
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GGG CAG CTG TCA GAC AGG TTT GGC CGG AAG AAT GTG CTG TTC GTG ACC 239

Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Val Thr
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Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln Ile
100 105 110

TCC AAC TAT GTG GCA GCA TTT GTC CTG GGG ACA GAA ATT CTT GGC AAG 383
Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly Lys
115 120 125

TCA GTT CGT ATA ATA TTC TCT ACG TTA GGA GTG TGC ATA TTT TAT GCA 431
Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr Ala
130 135 140

TTT GGC TAC ATG GTG CTG CCA CTG TTT GCT TAC TTC ATC CGA GAC TGG 479
Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp Trp
145 150 155

CGG ATG CTG CTG GTG GCG CTG ACG ATG CCG GGG GTG CTG TGC GTG GCA 527
Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val Ala
160 165 170 175

CTC TGG TGG TTC ATC CCT GAG ACC CCC CGA TGG CTC ATC TCT CAG GGA 575
Leu Trp Trp Phe Ile Pro Glu Thr Pro Arg Trp Leu Ile Ser Gln Gly
180 185 190

CGA TTT GAA GAG GCA GAG GTG ATC ATC CGC AAG GCT GCC AAA GCC AAT 623
Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala Asn
195 200 205

GGG GTT GTT GTG CCT TCC ACT ATC TTT GAC CCG AGT GAG TTA CAA GAC 671
Gly Val Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln Asp
210 215 220

CTA AGT TCC AAG AAG CAG CAG TCC CAC AAC ATT CTG GAT CTG CTT CGA 719
Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu Arg
225 230 235

ACC TGG AAT ATC CGG ATG GTC ACC ATC ATG TCC ATA ATG CTG TGG ATG 767
Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp Met
240 245 250 255

ACC TTA TCA GTG GGC TAT TTT GGG CTT TCG CTT GAT ACT CCT AAC TTG 815

Thr Leu Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn Leu
260 265 270
CAT GGG GAC ATC TTT GTG AAC TGC TTC CTT TCA GCG ATG GTT GAA GTC 863
His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu Val
275 280 285
CCA GCA TAT GTG TTG GCC TGG CTG CTG CTG CAA TAT TTG CCC CGG CGC 911
Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg Arg
290 295 300
TAT TCC ATG GCC ACT GCC CTC TTC CTG GGT GGC AGT GTC CTT CTC TTC 959
Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu Phe
305 310 315
ATG CAG CTG GTA CCC GTG GAC TTG TAT TAT TTG GCT ACA GTC CTG GTG 1007
Met Gln Leu Val Pro Val Asp Leu Tyr Tyr Leu Ala Thr Val Leu Val
320 325 330 335
ATG GTG GGC AAG TTT GGA GTC ACG GCT GCC TTT TCC ATG GTC TAC GTG 1055
Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr Val
340 345 350
TAC ACA GCC GAG CTG TAT CCC ACA GTG GTG AGA AAC ATG GGT GTG GGA 1103
Tyr Thr Ala Glu Leu Tyr Pro Thr Val Val Arg Asn Met Gly Val Gly
355 360 365
GTC AGC TCC ACA GCA TCC CGC CTG GGC AGC ATC CTG TCT CCC TAC TTC 1151
Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr Phe
370 375 380
GTT TAC CTT GGT GCC TAC GAC CGC TTC CTG CCC TAC ATT CTC ATG GGA 1199
Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met Gly
385 390 395
AGT CTG ACC ATC CTG ACA GCC ATC CTC ACC TTG TTT CTC CCA GAG AGC 1247
Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu Ser
400 405 410 415
TTC GGT ACC CCA CTC CCA GAC ACC ATT GAC CAG ATG CTA AGA GTC AAA 1295
Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val Lys
420 425 430
GGA ATG AAA CAC AGA AAA ACT CCA AGT CAC ACA AGG ATG TTA AAG GAT 1343
Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys Asp
435 440 445
GGT CAA GAA AGG CCC ACA ATC CTT AAA AGC ACA GCC TTC TAACATCGCT 1392

Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe
450 455 460

TCCAGTAAGG GAGAAACTGA AGAGGAAAGA CTGTCTTGCC AGAAATGGCC AGCTTGTGCA 1452
GACCGAGTCC TTCAGTGACA AAAGGCCTTT GCTGTTTGTC CTCTTGACCT GTGTCTGACT 1512
TGCTCCTGGA TGGGCACCCA CACTCAGAGG CTACATATGG CCC 1555

SEQ ID NO: 5
LENGTH: 22
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
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SEQ ID NO: 6
LENGTH: 21
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
TGTAGCGTGA AGACGACAGA A 21

SEQ ID NO: 7
LENGTH: 22
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
TCGAGCGGCC GCCCGGGCAG GT 22

SEQ ID NO: 8
LENGTH: 22
TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
AGGGCGTGGT GCGGAGGGCG GT 22

SEQ ID NO: 9
LENGTH: 20
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
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SEQ ID NO: 10
LENGTH: 24
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
AGAGGTGGCT TATGAGTATT TCTT 24

SEQ ID NO: 11
LENGTH: 22
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
CCAGGGTTTT CCCAGTCACG AC 22

SEQ ID NO: 12
LENGTH: 22
TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
TCACACAGGA AACAGCTATG AC 22

SEQ ID NO: 13
LENGTH: 24
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
GTGCTGTTGG GCTCCTTCAT TTCA 24

SEQ ID NO: 14
LENGTH: 24
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
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SEQ ID NO: 15
LENGTH: 24
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other synthetic DNA
SEQUENCE
AGCATCCTGT CTCCCTACTT CGTT 24

[Brief Description of the Drawings]

[Figure 1] Figure 1 represents the comparison of amino acid sequences of OCT family proteins. The initial letters of "h" indicates human-derived, "m" indicates mouse-derived, and "r" indicates rat-derived.

[Figure 2] Figure 2 represents hydrophobicity plots of OCT family proteins according to Kyte & Doolittle's calculating formula with a window of nine amino acid residues.

[Figure 3] Figure 3 represents electrophoretic patterns showing the results of Northern blot analysis of fls631.

[Figure 4] Figure 4 compares the fls631 similar sequence derived from q regions of human chromosome 5 (5q Genome) and fls631 sequence.

[Figure 5] Figure 5 compares the amino acid sequence of fls631 with that of 631RT. Sequences coinciding with the consensus sequences of sugar transporter and the ATP/GTP binding site are indicated by "+" and "*," respectively.

[Figure 6] Figure 6 represents electrophoretic patterns showing the results of Northern blot analysis of 631RT.

[Figure 7] Figure 7 is a graph showing the TEA-absorbing activity of fls631. Clear circles represent untreated cells, and solid circles represent fls631-transfected cells.

[Figure 8] Figure 8 is a graph showing effects of the cold TEA added in the experimental system in Fig. 7. In this graph, solid circles represent fls631-transfected cells, and clear circles represent cells containing the vector with no insert. Clear triangles indicate the net uptake induced by fls631 obtained by subtracting the clear circle values from the corresponding solid circle values.

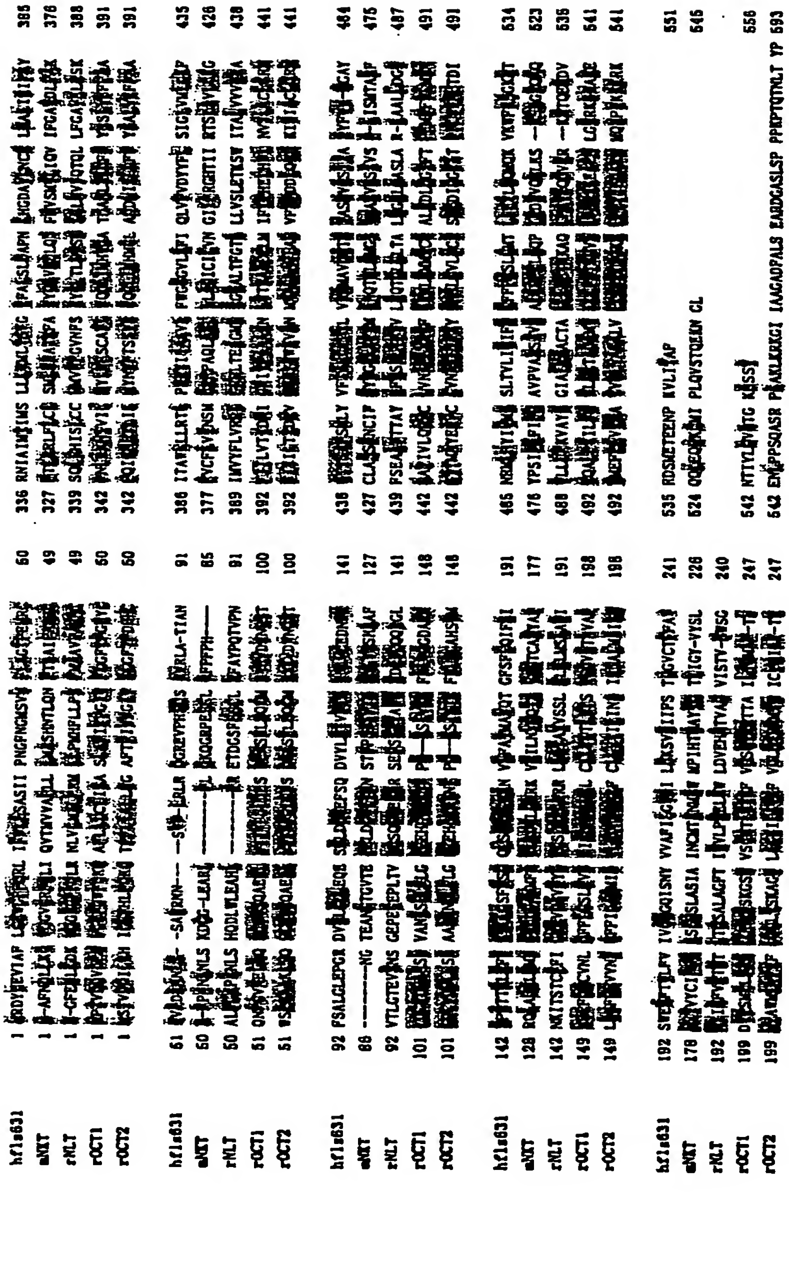
[Figure 9] Figure 9 is a graph showing TEA concentration-dependency of the TEA-absorbing activity of fls631.

[Figure 10] Figure 10 is a bar graph showing the activity of the fls631-transfected cells to absorb substances other than TEA.

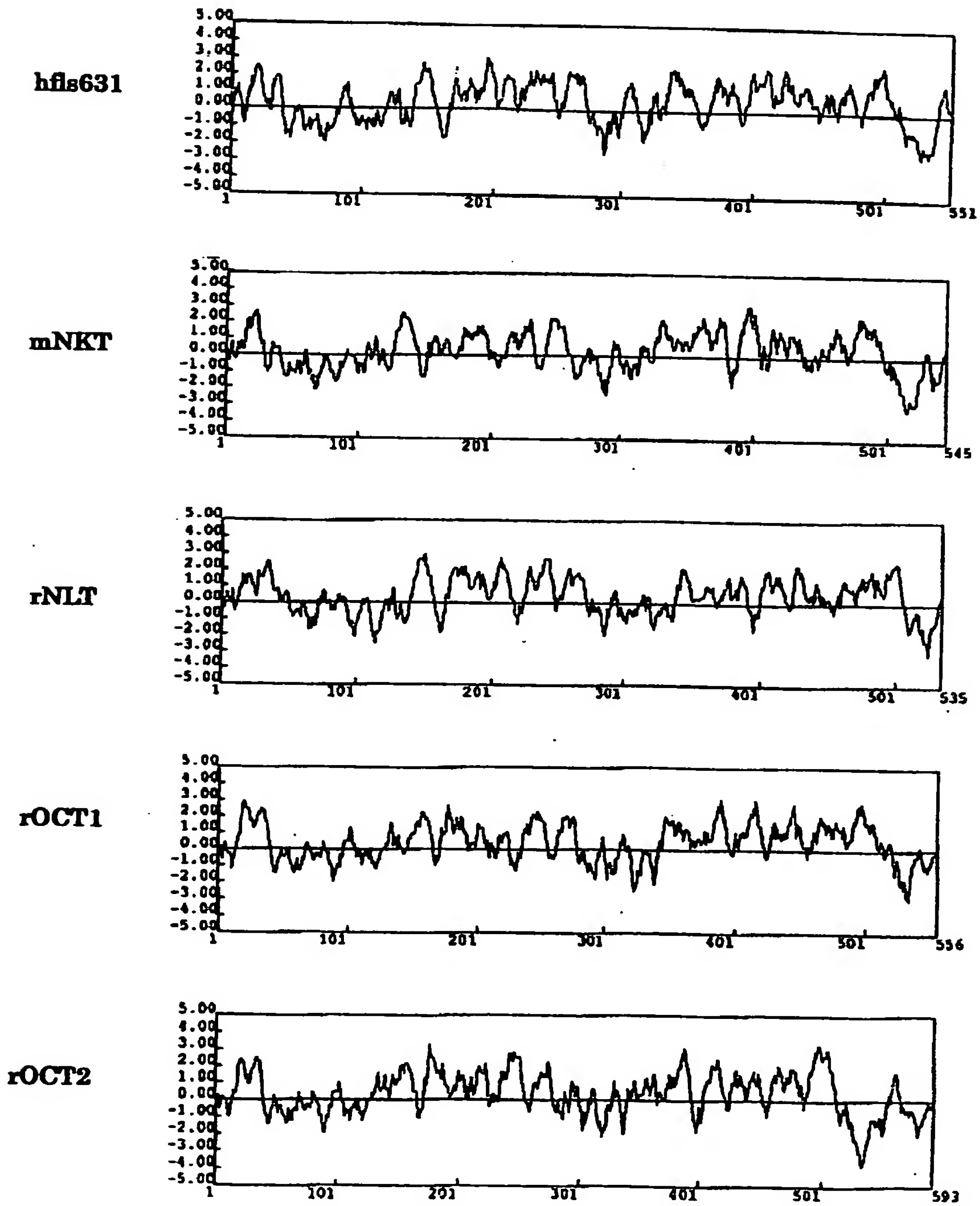
Reference No. = C2-906 (1/10)

Drawings

【Figure 1】



【Figure 2】


hfls631
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
1
101
201
301
401
501
551
mNKT
1
101
201
301
401
501
545
rNLT
1
101
201
301
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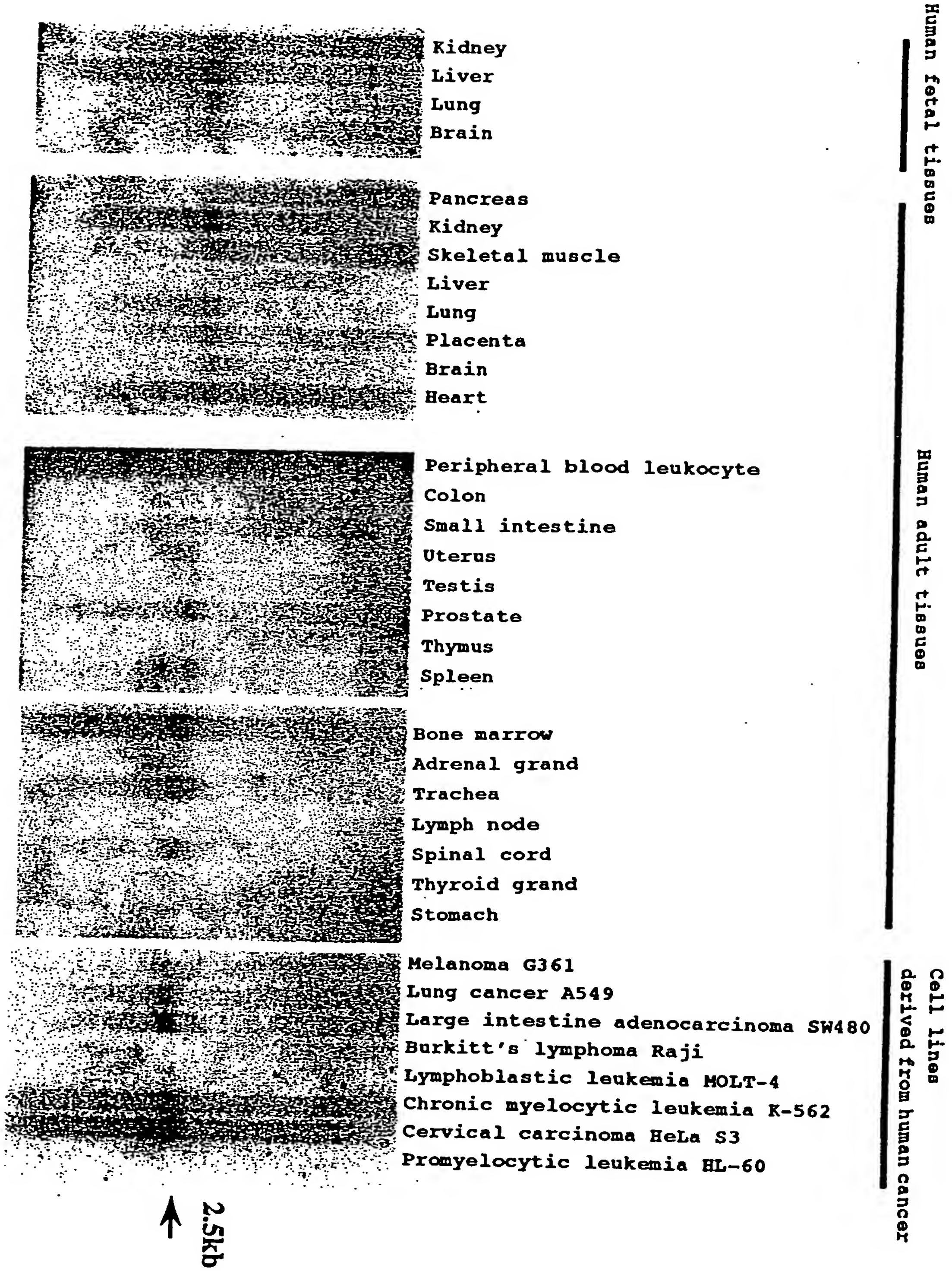
201
301
401
501
593

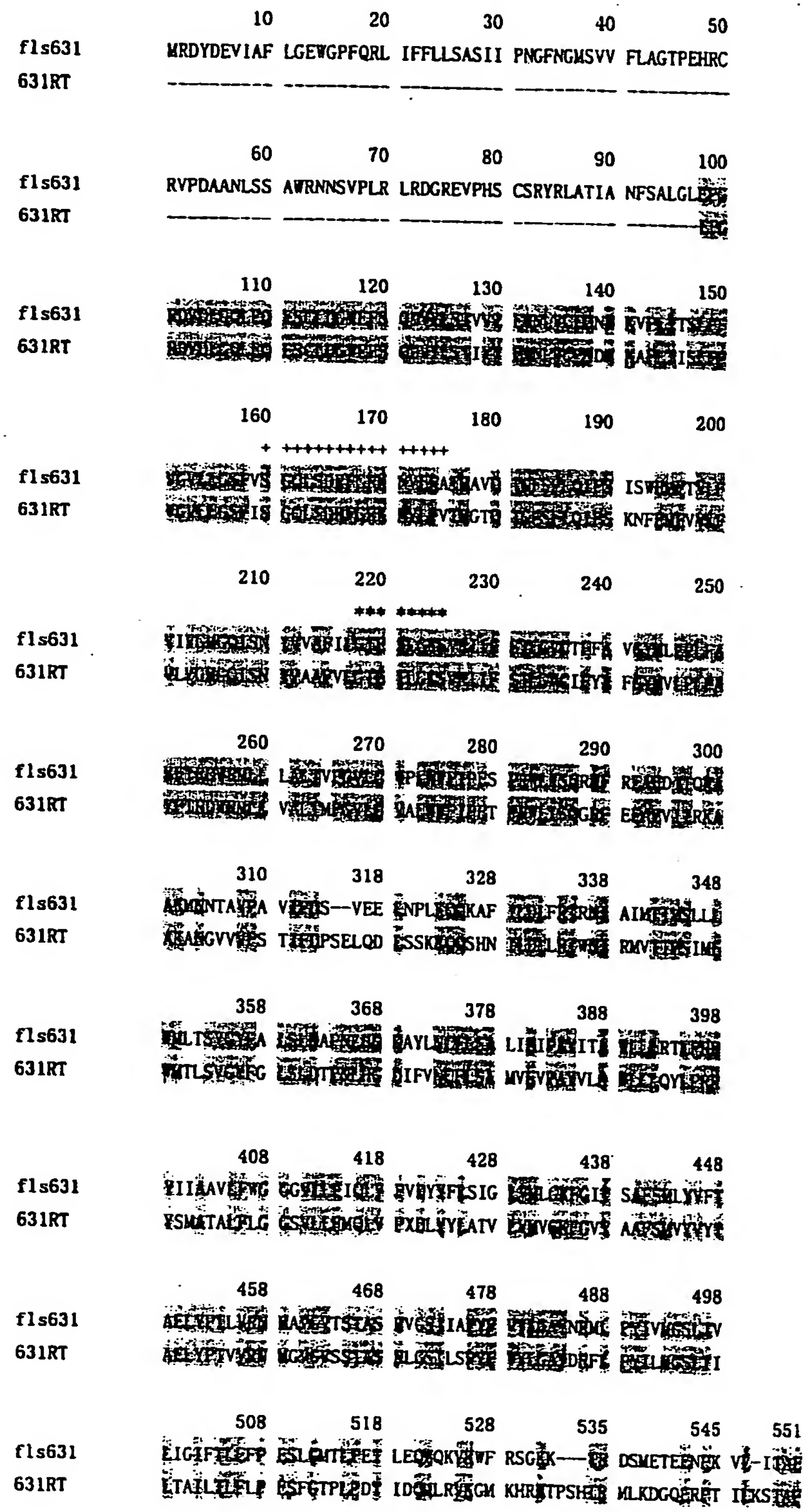

Kidney
Liver
Lung
Brain
Pancreas
Kidney
Skeletal muscle
Liver
Lung
Placenta
Brain
Heart
Peripheral blood leukocyte
Colon
Small intestine
Uterus
Testis
Prostate
Thymus
Spleen
Bone marrow
Adrenal grand
Trachea
Lymph node
Spinal cord
Thyroid grand
Stomach
Melanoma G361
Lung cancer A549
Large intestine adenocarcinoma SW480
Burkitt's lymphoma Raji
Lymphoblastic leukemia MOLT-4
Chronic myelocytic leukemia K-562
Cervical carcinoma HeLa S3
Promyelocytic leukemia HL-60
Human fetal tissues
Human adult tissues
Cell lines derived from human cancer
2.5kb

[Figure 3]

[Figure 4]

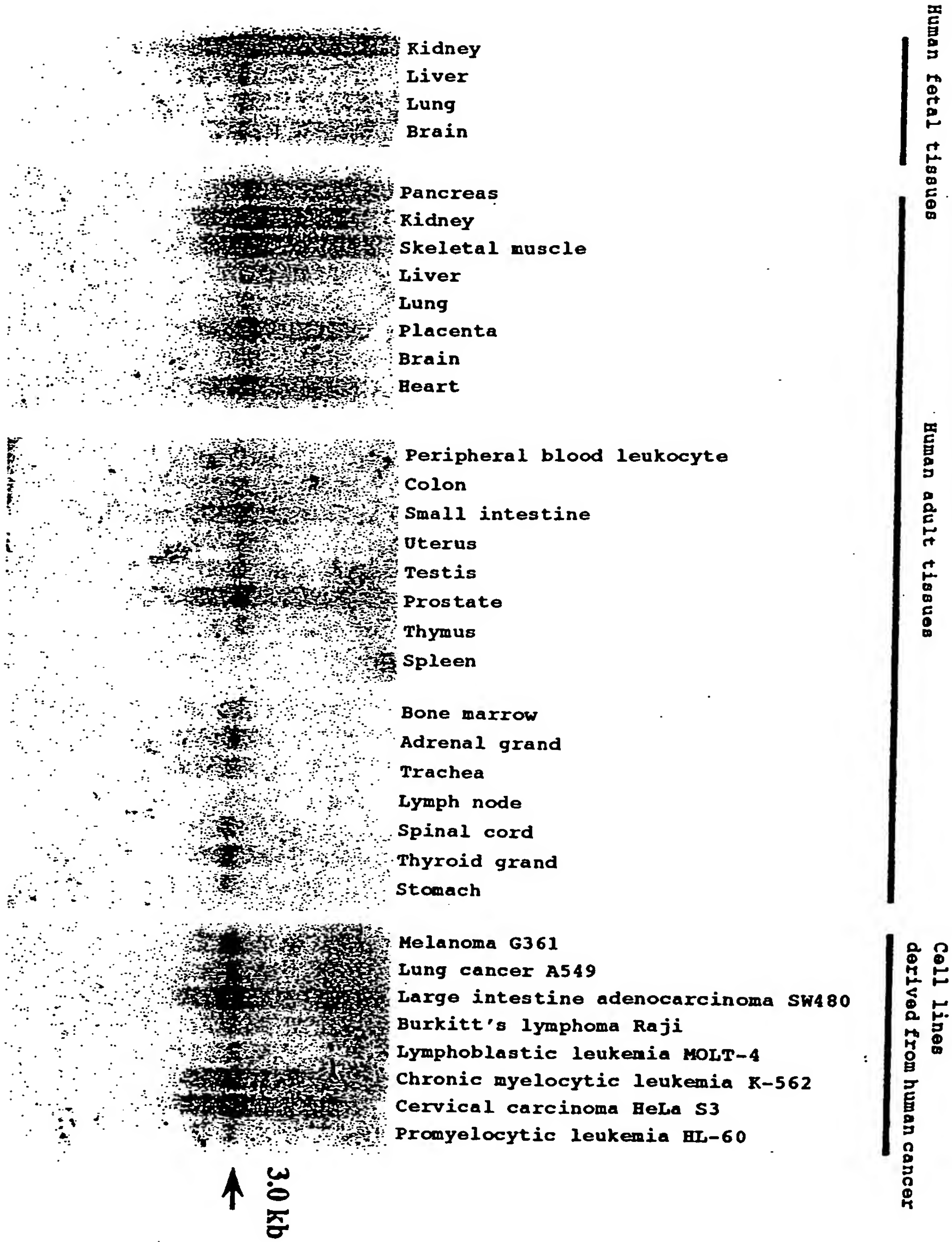
```
                    10        20        30        40        50        60
5q Genome   GGATGACCATATCAGTGGGCTATTTTGGGCTTTCGCTTGATACTCCTAACTTGCATGGGG
            X::::   :  :::::::: :: ::::  :: :: :: ::: ::::::: :: ::::: :
fls631      GGATGCTGACCTCAGTGGGTTACTTTGCTCTGTCTCTGGATGCTCCTAATTTACATGGAG
                  1200      1210      1220      1230      1240      1250

                    70        80        90       100       110       120
5q Genome   ACATCTTTGTGAACTGCTTCCTTTCAGCGATGGTTGAAGTCCCAGCATATGTGTTGGCCT
            :   ::   ::::::: ::::: :: ::  :: ::::: : ::::: ::  :    ::::
fls631      ATGCCTACCTGAACTGTTTCCTCTCTGCCTTGATTGAAATTCCAGCTTACATTACAGCCT
                  1260      1270      1280      1290      1300      1310
                   130       140       150       160       170       180
5q Genome   GGCTGCTGCTGCAATATTTGCCCCGGCGCTATTCCATGGCCACTGCCCTCTTCCTGGGTG
            :::::::  ::: :    ::::: :::: :::  ::: ::   : :  :: :::  ::: :
fls631      GGCTGCTATTGCGAACGCTGCCCAGGCGTTATATCATAGCTGCAGTACTGTTCTGGGGAG
                  1320      1330      1340      1350      1360      1370
                   190       200       210       220       230       240
5q Genome   GCAGTGTCCTTCTCTTCATGCAGCTGGTACCCNNNGACTTGTATTATTTGGCTACAGTCC
            :  :::: ::::::::::: :: :::::::: ...:: :  :: :  ::  : :  :  :
fls631      GAGGTGTGCTTCTCTTCATTCAACTGGTACCTGTGGATTATTACTTCTTATCCATTGGTC
                  1380      1390      1400      1410      1420      1430
                   250       260       270       280       290       300
5q Genome   TGGTGATGGTGGGCAAGTTTGGAGTCACGGCTGCCTTTTCCATGGTCTACGTGTACACAG
            :::: ::: :::: :: :::::  ::::  :::: :: :::::: : :: :: : ::: :
fls631      TGGTCATGCTGGGAAAATTTGGGATCACCTCTGCTTTCTCCATGCTGTATGTCTTCACTG
                  1440      1450      1460      1470      1480      1490
                   310       320       330       340       350       360
5q Genome   CCGAGCTGTATCCCACAGTGGTGAGAAACATGGGTGTGGGAGTCAGCTCCACAGCATCCC
            : ::::: :: :: ::  :::: :: :::::::  ::::: ::::  ::::: :: :::
fls631      CTGAGCTCTACCCAACCCTGGTCAGGAACATGGCGGTGGGGGTCACATCCACGGCCTCCA
                  1500      1510      1520      1530      1540      1550
                   370       380       390       400
5q Genome   GCCTGGGCAGCATCCTGTCTCCCTACTTCGTTTACCTTGGT
            :  ::::::::::: :  : :::::::: :::::::X :::
fls631      GAGTGGGCAGCATCATTGCCCCCTACTTTGTTTACCTCGGT
                  1560      1570      1580      1590
```

【Figure 5】



fls631
631RT
10 20 30 40 50
MRDYDEVIAF LGEWGPFQRL IFFLLSASII PNGFNGMSVV FLAGTPEHRC
60 70 80 90 100
RVPDAANLSS AWRNNSVPLR LRDGREVPHS CSRYRLATIA NFSALGL

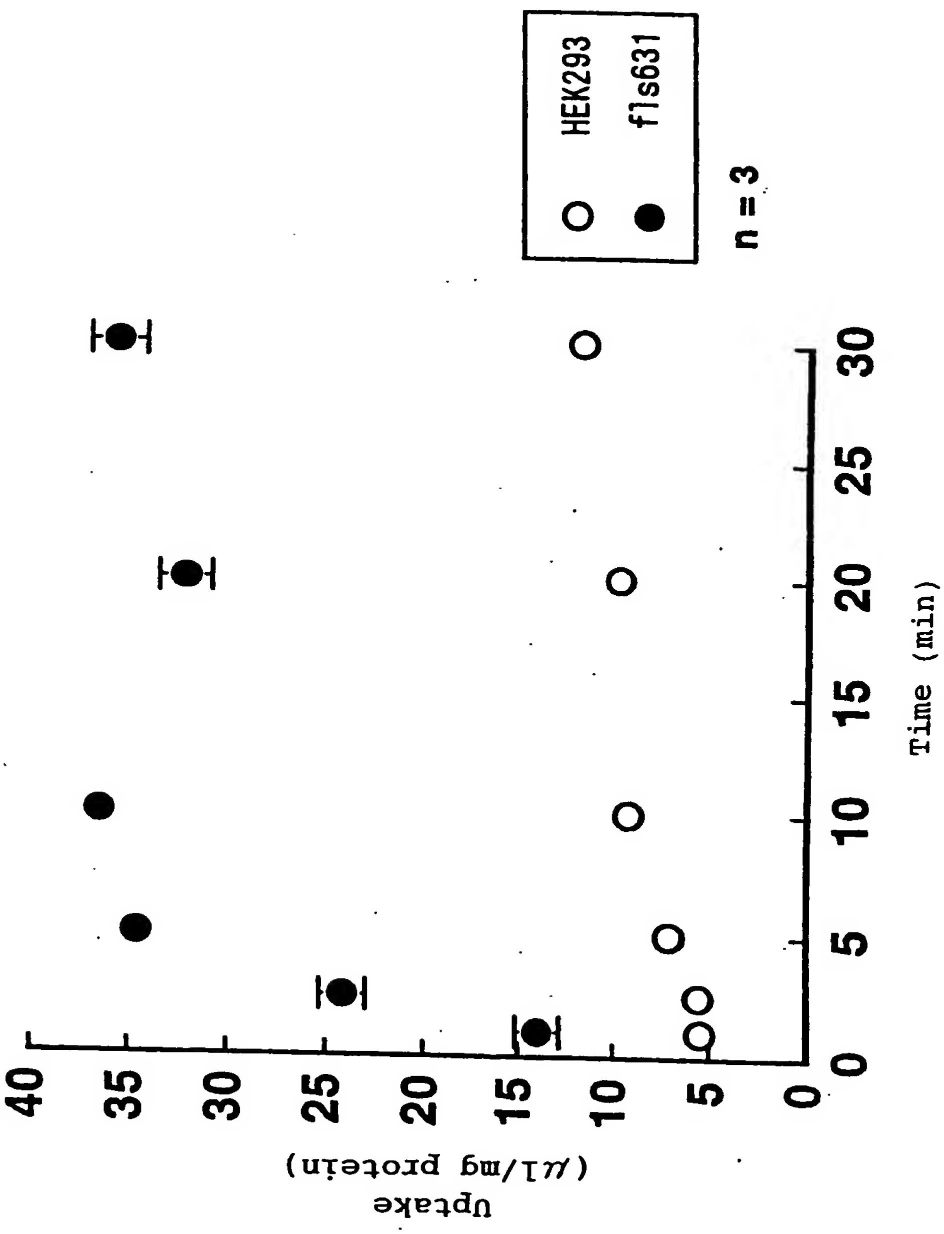
[Figure 6]



Reference No. = C2-906

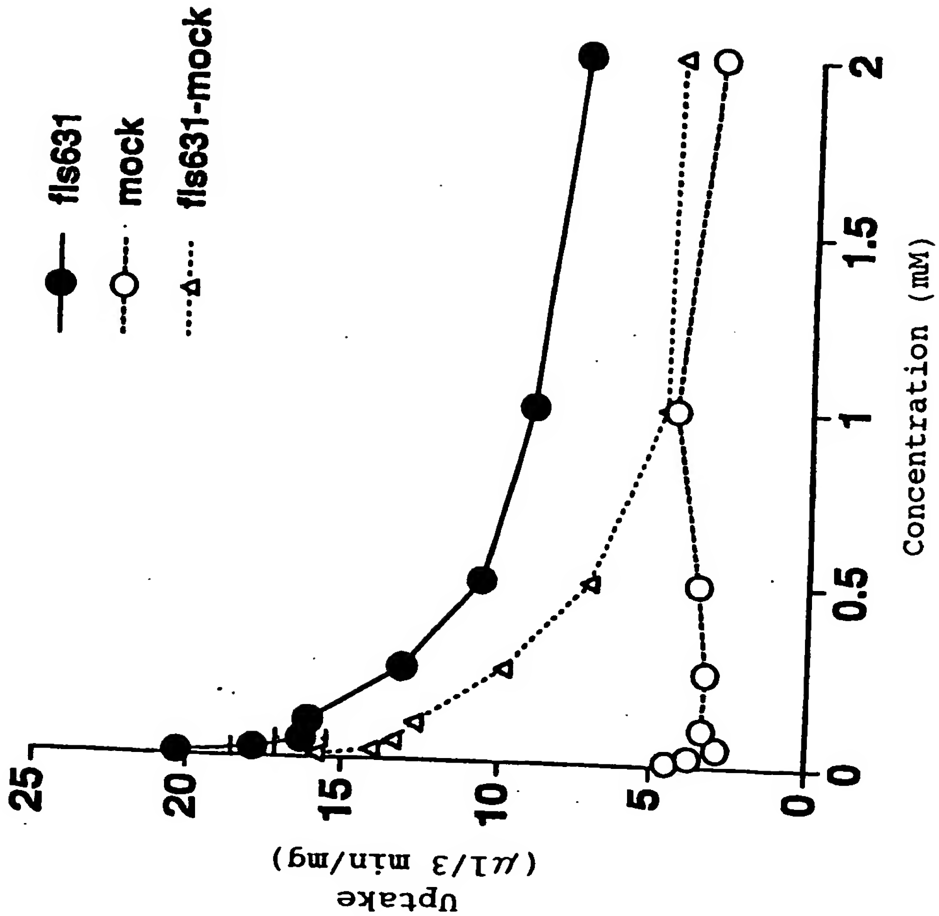
Reference No. = C2-906

【Figure 7】



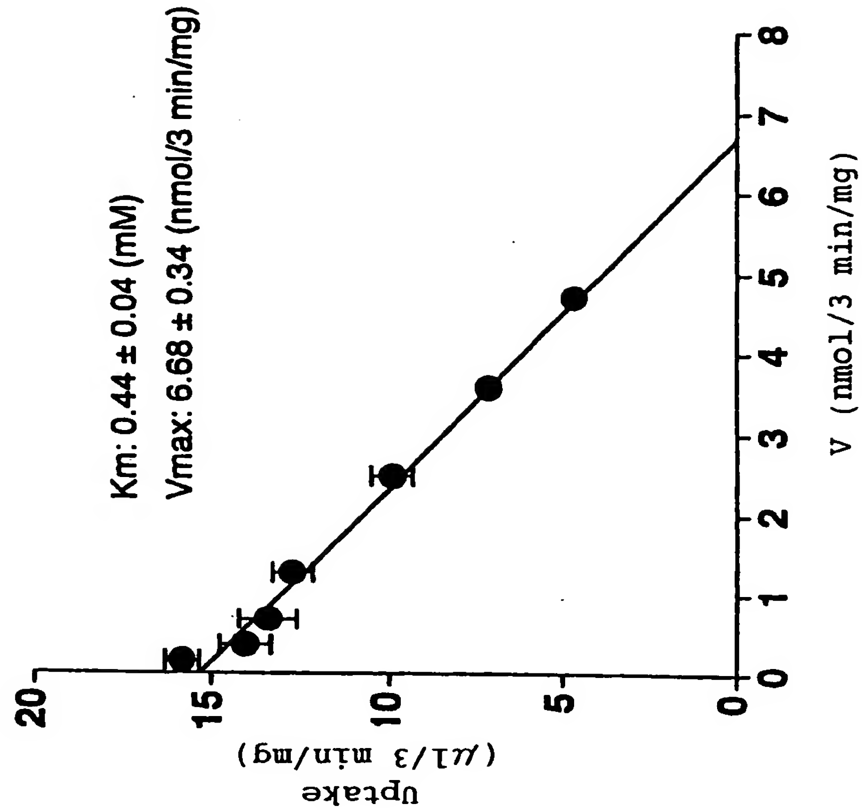
Reference No. = C2-906 (8/10)

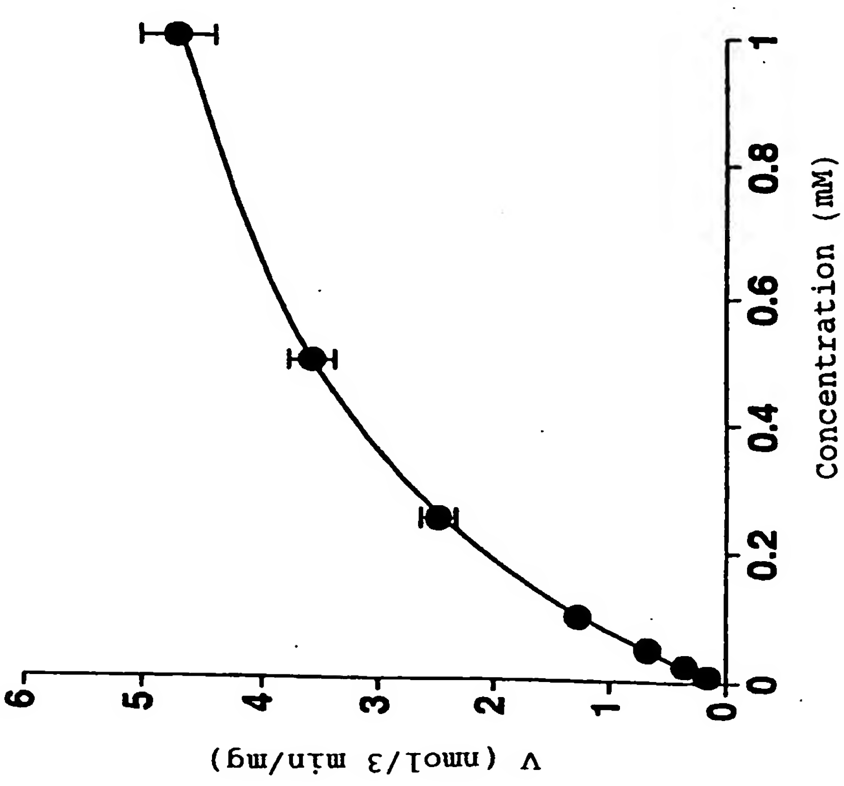
【Figure 8】



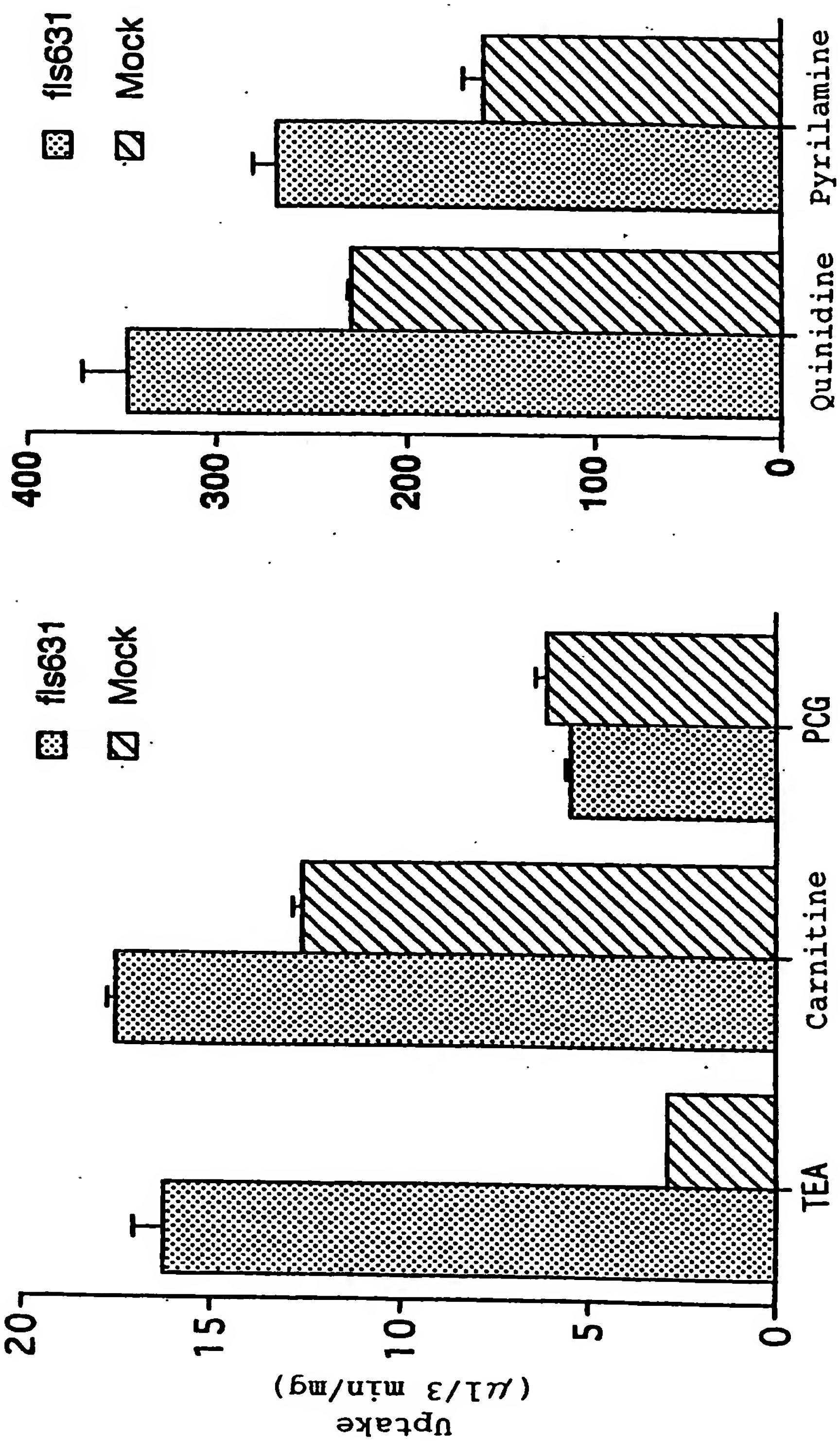
Reference No. = C2-906 (9/10)

【Figure 9】





【Figure 10】


fls631
Mock
Quinidine
Pyrilamine
400
300
200
100
0
fls631
Mock
TEA
Carnitine
PCG
20
15
10
5
0
Uptake
(μl/3 min/mg)

[Document Name] Abstract

[Abstract]

[Problems] The objects are to provide novel transporter genes and their proteins.

[Means of Solution] An unknown gene significantly homologous to known organic cation transporter family proteins has been successfully isolated by screening a fetal gene library using random sequencing. The inventors cloned the gene and analyzed its structure. As a result, it was found that the gene encodes a novel protein which has a high overall homology with organic cation transporter family proteins. In addition, after studying the transporter activity of the protein encoded by the isolated gene, it was revealed that the protein functions as a transporter of various organic cations.

[Selected Drawing] None